

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

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|---|--|--|-----------------------|
| Applicant's or agent's file reference JSB:SP:FP20705 | FOR FURTHER ACTION | | See Form PCT/IPEA/416 |
| International application No. PCT/AU2004/001577 | International filing date (day/month/year) 15 November 2004 | Priority date (day/month/year) 14 November 2003 | |
| International Patent Classification (IPC) or national classification and IPC | | | |
| Int. Cl. | CI2N 1/00 (2006.01) CI2N 1/20 (2006.01) | CI2N 1/26 (2006.01) CI2N 1/38 (2006.01) | |
| Applicant COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION et al | | | |

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

3. This report is also accompanied by ANNEXES, comprising:

a. ☒ (sent to the applicant and to the International Bureau) a total of 2 sheets, as follows:

☒ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).

☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

b. ☐ (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒ Box No. I Basis of the report

☐ Box No. II Priority

☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

☐ Box No. IV Lack of unity of invention

☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

☐ Box No. VI Certain documents cited

☐ Box No. VII Certain defects in the international application

☐ Box No. VIII Certain observations on the international application

| | |
|---|---|
| Date of submission of the demand 25 August 2005 | Date of completion of this report 14 February 2006 |
| Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929 | Authorized Officer GILLIAN ALLEN Telephone No. (02) 6283 2266 |

Box No. I Basis of the report

1. With regard to the language, this report is based on:
- ☒ The international application in the language in which it was filed
- ☐ A translation of the international application into _____, which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3(a) and 23.1 (b))
- ☐ publication of the international application (under Rule 12.4(a))
- ☐ international preliminary examination (Rules 55.2(a) and/or 55.3(a))
2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:
- ☐ the international application as originally filed/furnished
- ☒ the description:
- pages 1, 2, 4-62 as originally filed/furnished
- pages* 3 received by this Authority on 25-08-05 with the letter of 25-08-05
- pages* received by this Authority on with the letter of
- ☒ the claims:
- pages 64-66 as originally filed/furnished
- pages* as amended (together with any statement) under Article 19
- pages 63, received by this Authority on 14-12-05 with the letter of 14-12-05
- pages* received by this Authority on with the letter of
- ☒ the drawings:
- pages 1/12/-12/12; as originally filed/furnished
- pages* received by this Authority on with the letter of
- pages* received by this Authority on with the letter of
- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to the sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to the sequence listing (*specify*):

* If item 4 applies, some or all of those sheets may be marked "superseded."

Box No. III **Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ Part claims Nos 1-16, 18, 19, 21-23 and 25 and claims 17, 20 and 24

because:

☐ the said international application, or the said claims Nos.

relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos.
are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos.
are so inadequately supported by the description that no meaningful opinion could be formed (*specify*)

☒ no international search report has been established for said claim Nos. Part claims 1-16, 18, 19, 21-23 and 25 and claims 17, 20 and 24

☐ A meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ Furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ Furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ Pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13ter.1(a) or (b) and 13ter.2.

☐ A meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-bis of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.

☐ See Supplemental Box for further details.

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

| | | | |
|-------------------------------|--------|---------------------------------|-----|
| Novelty (N) | Claims | Part 1-16, 18, 19, 21-23 and 25 | YES |
| | Claims | | NO |
| Inventive step (IS) | Claims | | YES |
| | Claims | Part 1-16, 18, 19, 21-23 and 25 | NO |
| Industrial applicability (IA) | Claims | 1-25 | YES |
| | Claims | | NO |

2. Citations and explanations (Rule 70.7)**Citations**

- D1 Acha, V.; Meurens, M.; Naveau, H.; Agathos, S. N. Detoxification of a mixture of aliphatic chlorinated hydrocarbons in a fixed-bed bioreactor : continuous on-line monitoring via an attenuated total reflection-Fourier transform infrared sensor. Water Science and Technology (1999), 40(8), 41-47.
- D2 Stuart S L; Woods S L. Kinetic evidence for pentachlorophenol-dependent growth of a dehalogenating population in a pentachlorophenol- and acetate-fed methanogenic culture. Biotech and Bioeng, 1998. 57(4): 420-429.
- D3 Bellco CellTrol II Control Modules
<http://www.bellcoglass.com/us/7803-81102.shtml>. 31 August 2003. <http://www.archive.org/> used to establish the publication date of the document.
- D4 BioNet Utility Tower (Single, Dual, or Quad)
<http://www.broadleyjames.com/bionet-tower.html>. 2 October 2003. <http://www.archive.org/> used to establish the publication date of the document.

New Citation

- D5 Granato M et al. Biological treatment of a synthetic gold milling effluent. Environmental Pollution, 1996. 91(3): 343-350

Novelty and Inventive Step

The claims have been limited by Article 34 amendment. The present claims are directed to methods of selectively enriching microorganisms to metabolise a test substrate, wherein assessment of enrichment is monitored using a "signal indicative of the level of a metabolism indicator which is a terminal electron acceptor". An International Search was not performed on the invention as defined by the present claims, see Supplemental Box 1. The present claims have not been searched, and the documents selected on the basis of the original claims have limited relevance to the present claim set.

The opinion on novelty and inventive step is offered only in so far as the cited documents from the International Search are relevant to the present claims.

Continued in supplemental Box 2

Supplemental Box 1

In case the space in any of the preceding boxes is not sufficient.

Continuation of: **Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The original claims on which the International Search was established were directed to methods of selectively enriching microorganisms to metabolise a test substrate, wherein assessment of enrichment was monitored using a "signal indicative of the level of a **metabolism indicator**". The term "metabolism indicator" is a very broad term covering a large range of possible indicators.

The present claims are directed to methods of selectively enriching microorganisms to metabolise a test substrate, wherein assessment of enrichment is monitored using a "signal indicative of the level of a **metabolism indicator which is a terminal electron acceptor**".

The International Search was broadly directed to assessing the novelty of monitoring microbial enrichment using signals indicative of metabolism indicators. It was not focussed on metabolism indicators that are terminal electron acceptors. Thus, all features of the present claims were not searched.

A strictly limited opinion is provided on the novelty and inventiveness of the present claim set. The opinion is offered only in so far as the metabolism indicator is oxygen, and only in so far as the cited prior art covers the use of oxygen as a metabolism indicator.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: **Novelty and Inventive Step.**

The only citation relevant to the present claims is D5.

This citation does not disclose all features of claims 1-16, 18, 19, 21-23 and 25, in so far as they are limited to monitoring oxygen levels.

Therefore all claims are novel.

This citation discloses enrichment cultures set up to determine treatment efficiency of cultures of sewage sludge set up to detoxify gold mining effluent. The treatment efficiency was measured by monitoring dissolved oxygen, among other parameters. Oxygen is a terminal electron acceptor for most aerobic microorganisms. The citation states (p 349, col 1 para 2) that dissolved oxygen concentration is a parameter with significant influence on the biomass growth, which is related to efficiency of detoxification. It is noted that the citation states that dissolved oxygen was maintained in the bioreactor at a preset level throughout the test runs which lasted several weeks. Clearly this could be most readily achieved by regular or continuous monitoring, and adjusting oxygen/air flow (a fluid flow) based on the monitored results. Essentially the oxygen input required to maintain a desired dissolved oxygen level provides a measurement of oxygen use.

Although the citation does not disclose how oxygen levels were monitored, the use of oxygen electrodes to monitor dissolved oxygen concentration is standard methodology, and lends itself to electronic output and online monitoring.

Oxygen appears to be required for the metabolism of cyanide and thiocyanates present in the test effluent of the citation, and these molecules are regarded as not commonly metabolised substrates. Visual monitoring is standard. Selective pressure is a normal method of obtaining enrichment cultures, and one of skill in the art would expect to set and adjust experimental parameters to provide optimal conditions.

It is considered that claims 1-16, 18, 19, 21-23 and 25, in so far as they are directed to monitoring enrichment cultures by monitoring oxygen levels, lack inventive step over D5.

the enrichment of an enzyme produced by the microorganism that is involved in the metabolism of the first substrate.

Where the microorganism produces an enzyme, or enzymes, that are involved in the metabolism of the test substrate, the method enables the selective enrichment of a microorganism that produces such enzyme or enzymes.

The present inventors have found that the above method for "on-line" determination of a change in the level of a metabolism indicator, such as O_2 , as an indicator of cellular activity enables indirect measurement of biomass or substrate utilisation and have identified that this can be used to evaluate the status of a population of microorganisms in real-time. The inventors have further tailored this technique for enriching microorganisms that are capable of metabolising a test substrate, such as a hydrocarbon compound for which a microorganism is desired to be found to convert the compound (test substrate) into a different hydrocarbon(s) and/or break the compound down with water as a byproduct. Such metabolism may be accompanied by the production, or up-regulation of an enzyme or enzymes that are involved in the metabolism of the test substrate. Thus, the metabolism of the microorganism also reflects an increase in the population or amount of enzyme in the vessel (compared to the relative amount of that enzyme in the vessel at the outset of the procedure) that has the desired function of catalysing the reaction of the test substrate.

The technique developed by the inventors has further advantages in terms of its flexibility in discovering microorganisms capable of metabolising a test substrate in conditions selected by the operator (i.e. a selective pressure), and potentially modified by the operator over time. The modification of conditions can be used to identify microorganisms that have the capability of producing an enzyme or enzymes that assist in the metabolism of the test substrate under such conditions.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for selectively enriching for a microorganism able to metabolise a test substrate, and/or the enrichment of an enzyme involved in the metabolism of the test substrate, the method comprising the steps of
 - a) providing a population of microorganisms in a vessel,
 - b) feeding fluid into the vessel at a controlled flow rate commencing with an initial flow rate, the fluid comprising a nutrient medium and, for at least part of the feed period, the test substrate,
 - c) producing a signal indicative of the level of a metabolism indicator which is a terminal electron acceptor, over the time-frame of the enrichment, wherein the signal is produced from a probe that takes a reading in the vessel, and
 - d) providing an output showing the change in level of the metabolism indicator which is based on the signal of the probe to enable assessment of selective enrichment of a microorganism that metabolises the test substrate, and/or the enrichment of an enzyme produced by the microorganism that is involved in the metabolism of the test substrate.
2. The method of claim 1, wherein the output is produced electronically directly from the signal, such that the output is provided on-line.
3. The method of claim 1 or claim 2, wherein the method further comprises presetting conditions to be met by the signal output to result in a change in the fluid flow rate, and changing the flow rate at which fluid is fed into the vessel when the conditions are met, wherein the preset conditions are a combination of a predetermined period of time and a preset value range within which the signal must remain for the predetermined period of time.